Miao et al., Afr J Tradit Complement Altern Med. (2016) 13(2):52-60
http://dx.doi.org/10.4314/ajtcam.v13i2.7

EVALUATION OF ANTI-FATIGUE ACTIVITY OF FLAVONOIDS FROM TARTARY BUCKWHEAT IN MICE

Fusheng Miao*, D Wu, Guichao Ni

Liaoning Normal University, Dalian 116000, P.R. China
***Corresponding author**: **E-mail:** fsmunv@sina.com

Abstract

Background: Flavonoids are the major biological activities components of tartary buckwheat which has multifunctional bioactivities. However, there are a limited number of studies on the effect of flavonoids from tartary buckwheat (TBF) on physical fatigue at present. This study aimed to investigate the anti-fatigue activity of TBF in mice.

Materials and Methods: The mice were divided into four groups: control (C), low-dose TBF-treated (LFT), middle-dose TBF-treated (MFT) and high-dose TBF-treated (HFT). The treated groups received TBF (100, 200 and 400 mg/kg), while the control group received physiological saline. After 28 days' treatment, the mice performed exhaustive running exercise on the treadmill, along with the measure of exhaustive running times, blood lactic acid (BLA), serum urea nitrogen (SUN), serum creatine kinase (SCK), liver glycogen, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Results: TBF prolonged the exhaustive running time of the mice. It sub-served to remove the accumulated products of metabolism by decreasing the levels of BLA and SUN. It ameliorated the muscle damage by decreasing the SCK levels. It improved the metabolic control of exercise and activated the energy metabolism by increasing the liver glycogen contents, as well as improving endogenous cellular antioxidant enzymes in mice by increasing the SOD, GPx and CAT activities.

Conclusion: TBF has significant anti-fatigue activity.

Key words: anti-fatigue, flavonoids, tartary buckwheat, exhaustive running exercise, mice

Introduction

Fatigue is best defined as a condition or phenomenon of declined ability and deficiency of mental and/or physical activities caused by excessive mental or physical activities, or illness (Tanaka et al., 2013). Fatigue, especially physical fatigue, accelerates the obviously vascular structural changes and development of atherosclerosis. Furthermore, long-term accumulated fatigue can lead to karoshi (death from overwork), which is the major cause of death in the white-collar workers (Lin et al., 2014). Delayed occurrence of fatigue and quick recovery are current foci of medical chemistry studies. Clinical drugs used to relieve fatigue are limited, and potential alternatives from natural substances are worth investigating (Horng et al., 2014). Recently, it has been reported that some natural substances are effective in preventing or reducing fatigue (Tan et al., 2013; Yong-xin and Jian-jun, 2013; Li et al., 2013; Qi et al., 2014).

Tartary buckwheat (*Fagopyrum esculentum*) grain is an important functional food material. It contains proteins with high biological value and balanced amino acid composition, relatively high crude fiber and vitamins B_1, B_2, and B_6, and more flavonoids than common buckwheat (Fabjan et al., 2003; Zhang et al., 2010). Previous studies on biochemical activities from tartary buckwheat mainly focused on its flavonoids (quercetin 3-O-rutinoside, quercetin 3-O-rutinoside-3'-O-β-glucopyranoside, kaempferol-3-O-rutinoside, and quercetin) which included antioxidant, hypoglycemic, hypolipidemic, antihypertensive, anti-inflammatory, and anti-tumour activities (Gong et al., 2012). However, there are a limited number of studies on the effect of

flavonoids from tartary buckwheat (TBF) on physical fatigue at present. Thus, the present study was undertaken to assess the anti-fatigue activity of TBF in mice.

Materials and Methods

Plant Material and Reagents

The air-dried tartary buckwheat grain was purchased from Liangshan in Sichuan Province and identified by Dr. Wang H.M. (School of Life Sciences, Liaoning Normal University, Dalian, China). The identity was confirmed by comparing with a voucher specimen available in the herbarium of Liaoning Normal University. The dried tartary buckwheat grain was ground to fine powder and then stored in airtight glass jars at 4 ℃ until being used. The detection kits of blood lactic acid (BLA), serum urea nitrogen (SUN), liver glycogen, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were purchased from Jiancheng Science & Technology Ltd. (Nanjing, China). The detection kit of serum creatine kinase (SCK) was purchased from Jimi Biotechnology Co., Ltd. (Shanghai, China). All other reagents used in this study were analytical grade and were obtained locally.

Animals

Male Kun-ming (KM) mice weighing 20 ± 2 g were purchased from Experimental Animal Center of Liaoning Normal University [Certificate No: LNXK 2014-0089]. The animals were acclimatized for one week prior to the experiment, during which they were given free access to water and standard mouse food, which consisted of 10% fat, 64% carbohydrate, and 26% protein. The mice were housed in a room maintained at 23 ± 2℃ with relative air humidity of 50 ± 5 % on a 12 h light-dark cycle. All animal handling procedures were performed in strict accordance with the use and care of laboratory animals of the PR. China legislation and were approved by the Animal Ethics Committee of Liaoning Normal University (No. ANPZ 2014062).

Preparation of Flavonoids from Tartary Buckwheat

The method of Gong et al. (2012) was used in the preparation of flavonoids from tartary buckwheat (TBF). The procedures are described as follows: The sample powder (10 g) was subjected to hot continuous extraction in Erlenmeyer flask with ethanol (ethanol concentration 60%, ratio of solvent to raw material 40), and the temperature of the water bath was 70 ℃. After 2.0 h, the extract was filtered through the filter. The obtained filtrate was evaporated by using a rotary evaporator to crude TBF. The flavonoid content was measured to be 25.43 mg/g using a modified colorimetric method (Jia et al., 1999).

Experimental Design

The mice were randomly divided into four groups, each containing 12 animals as follows: control (C) group, low-dose TBF-treated (LFT) group, middle-dose TBF -treated (MFT) group and high-dose TBF-treated (HFT) group. The mice in the treated groups received TBF (100, 200 and 400 mg/kg, respectively) intragastrically (ig) for 28 days and TBF were dissolved in 1.5 mL of physiological saline. The mice in the control group received the same volume of physiological saline ig for 28 days.

The mice were instructed to treadmill running with 15 min exercise bouts at 20 m/min and a slope of 5% for a week to be accustomed to running. On the last day of treatment, the mice were required to run to exhaustion on the treadmill (rat/mouse treadmill, Columbus, OH, USA) at 32 m/min and a slope of 8 %. Mice were made to run at this speed and slope until they were unable to respond to continued prodding with a soft brush (Azenabor and Hoffman-Goetz, 1999). The exhaustive running times were recorded.

Biochemical Analysis

Immediately after exhaustive exercise, the mice were killed under diethyl ether anesthesia, and blood samples were collected in

heparinized tubes for BLA, SUN and SCK analysis. Then, the livers were removed and then frozen in liquid N_2 for glycogen, SOD, CAT, GPx and MDA analysis. All the biochemical parameters were measured using respective detection kits according to the manufacturers' instructions.

Statistical Analysis

The data are expressed as mean ± SD for all the groups. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test for evaluating differences between groups using SPSS 13.0 (v.13.0; SPSS Inc., Chicago, III, USA). $P<0.05$ was considered as statistically significant.

Results

Effect of TBF on Exhaustive Running Times of Mice

Figure 1 demonstrates the effect of TBF on the exhaustive running times of mice. Compared with that of the C group, the exhaustive running time of the LFT, MFT and HFT groups were significantly longer ($P<0.05$).

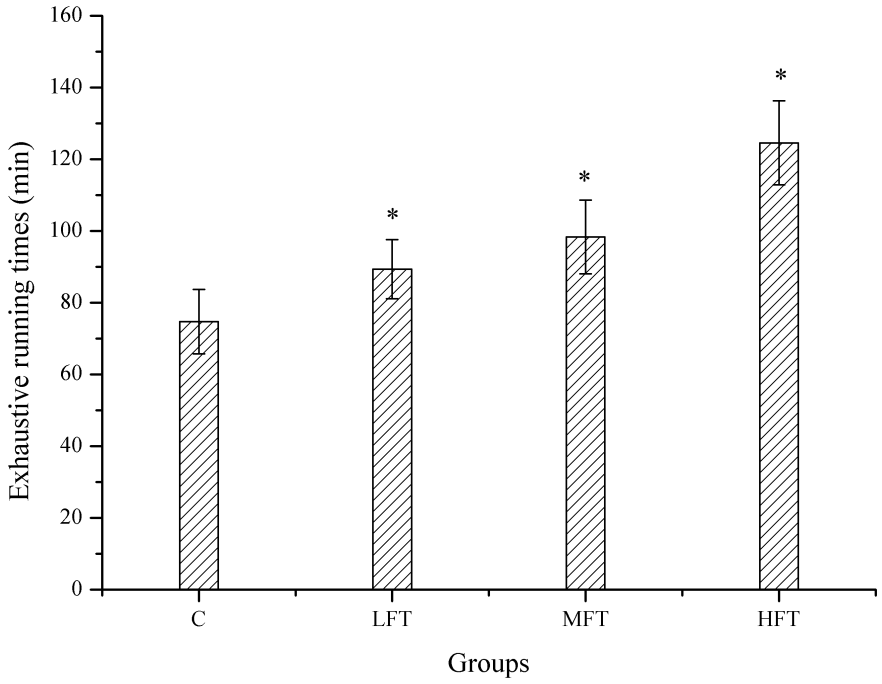

Figure 1: Effect of TBF on exhaustive running times of mice. Data are mean ± SD (n = 12 mice per group). *P<0.05 vs Control. C: control; LFT: low-dose TBF-treated (100 mg/kg); MFT: middle-dose TBF-treated (200 mg/kg); HFT: high-dose TBF-treated (400 mg/kg).

Effect of TBF on Blood Lactic Acid of Mice

Figure 2 demonstrates the effect of TBF on the blood lactic acid (BLA) of mice. Compared with those of the C group, the BLA levels of the LFT, MFT and HFT groups were significantly lower ($P<0.05$).

http://dx.doi.org/10.4314/ajtcam.v13i2.7

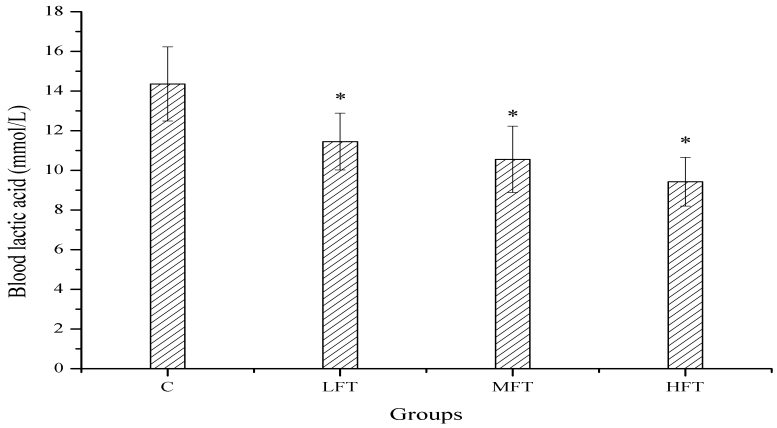

Figure 2: Effect of TBF on blood lactic acid of mice. Data are mean ± SD (n = 12 mice per group). *P<0.05 vs Control. C: control; LFT: low-dose TBF-treated (100 mg/kg); MFT: middle-dose TBF-treated (200 mg/kg); HFT: high-dose TBF-treated (400 mg/kg).

Effect of TBF on Serum Urea Nitrogen of Mice

Figure 3 demonstrates the effect of TBF on the serum urea nitrogen (SUN) of mice. Compared with those of the C group, the SUN levels of the MFT and HFT groups were significantly lower (P<0.05); although the SUN levels of the LFT group were also lower, but not significantly (P>0.05).

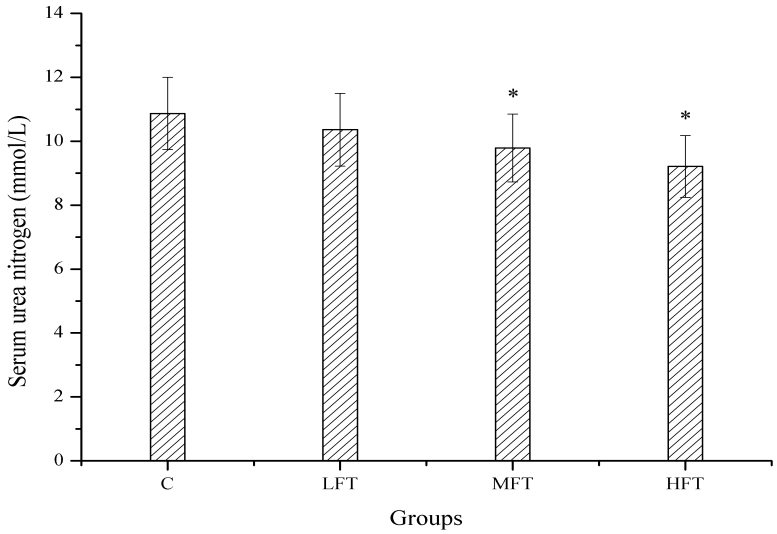

Figure 3: Effect of TBF on serum urea nitrogen of mice. Data are mean ± SD (n = 12 mice per group). *P<0.05 vs Control. C: control; LFT: low-dose TBF-treated (100 mg/kg); MFT: middle-dose TBF -treated (200 mg/kg); HFT: high-dose TBF-treated (400 mg/kg).

Effect of TBF on Serum Creatine Kinase of Mice

Figure 4 demonstrates the effect of TBF on the serum creatine kinase (SCK) of mice. Compared with the C group, the SCK

http://dx.doi.org/10.4314/ajtcam.v13i2.7

levels of the HFT groups were significantly lower (P<0.05); although the SCK levels of the MFT and LFT group was also lower, but not significantly (P>0.05).

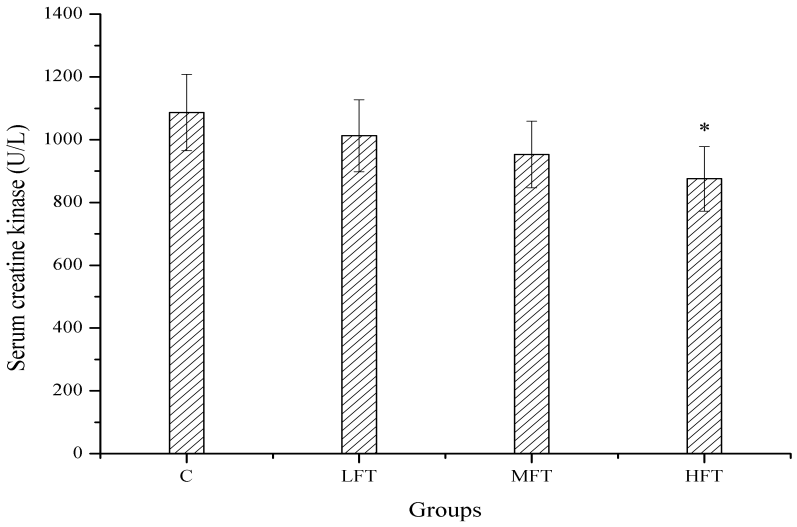

Figure 4: Effect of TBF on serum creatine kinase of mice. Data are mean ± SD (n = 12 mice per group). *P<0.05 vs Control. C: control; LFT: low-dose TBF-treated (100 mg/kg); MFT: middle-dose TBF -treated (200 mg/kg); HFT: high-dose TBF-treated (400 mg/kg).

Effect of TBF on Liver Glycogen of Mice

Figure 5 demonstrates the effect of TBF on the liver glycogen of mice. Compared with those of the C group, the liver glycogen contents of the LFT, MFT and HFT groups were significantly higher (P<0.05).

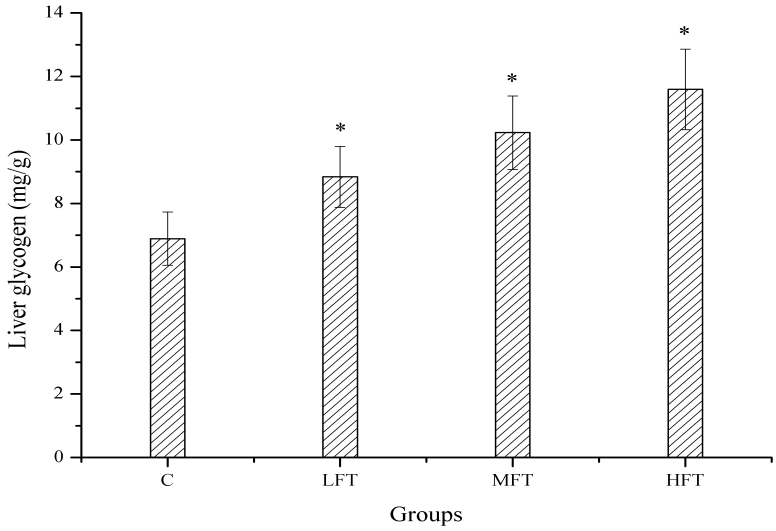

Figure 5: Effect of TBF on liver glycogen of mice. Data are mean ± SD (n = 12 mice per group). *P<0.05 vs Control. C: control; LFT: low-dose TBF-treated (100 mg/kg); MFT: middle-dose TBF -treated (200 mg/kg); HFT: high-dose TBF-treated (400 mg/kg).

http://dx.doi.org/10.4314/ajtcam.v13i2.7

Effect of TBF on Antioxidant Enzymes in Liver of Mice

Figure 6 demonstrates the effect of TBF on the antioxidant enzymes in liver of mice. Compared with those of the C group, the SOD and CAT activities in liver of the LFT, MFT and HFT groups were significantly higher ($P<0.05$). The GPx activities in liver of the MFT and HFT groups were significantly higher ($P<0.05$); The GPx activities in liver of the LFT group was also higher, but not significantly ($P>0.05$).

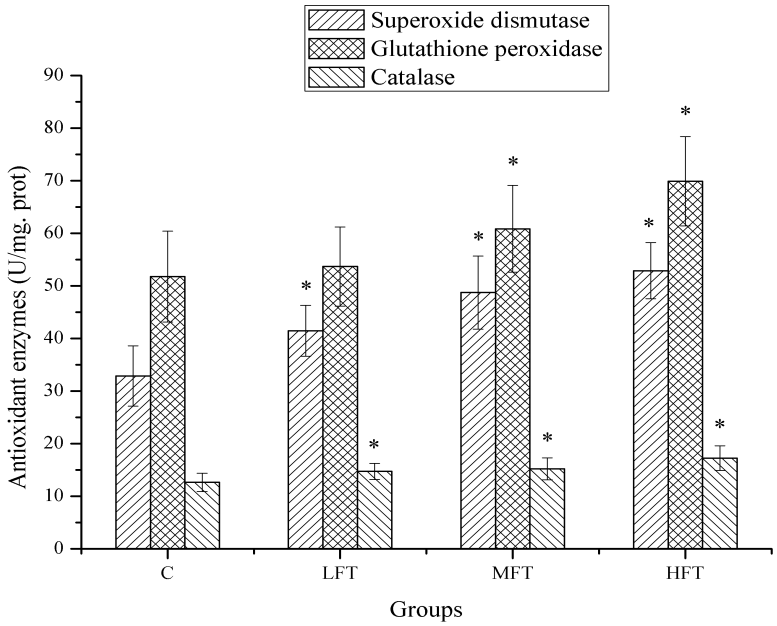

Figure 6: Effect of TBF on antioxidant enzymes of mice. Data are mean ± SD (n = 12 mice per group). *P<0.05 vs Control. C: control; LFT: low-dose TBF-treated (100 mg/kg); MFT: middle-dose TBF -treated (200 mg/kg); HFT: high-dose TBF-treated (400 mg/kg).

Discussion

The anti-fatigue activity can be evaluated directly by measuring the increase in exercise tolerance and endurance capacity in mice (Sun et al., 2014). In this study, treadmill running to exhaustion is an experimental exercise model to evaluate anti-fatigue, which can reflect the fatigue degree of movement and objectively reflect the physical ability of body (Ding et al., 2011). In this study, the data showed that the exhaustive running time of the LFT, MFT and HFT groups were significantly longer compared with that in the control group. The results clearly indicated that TBF has anti-fatigue activity and can elevate the exercise tolerance.

Lactic acid is the glycolysis product of carbohydrate under anaerobic conditions, and glycolysis is the main energy source for intense exercise in a short time (Ding et al., 2011). The increased lactic acid production results in the decrease of the internal pH value, which may lead to impairment of muscle contraction and harm organs, and also causes fatigue (Qi et al., 2014). Blood lactic acid (BLA) level is dependent on the rate of lactate production by glycolysis and its utilization as a substrate, and lactate production increases with the degree of exercise intensity (Takeda et al., 2011). Consequently, BLA is one of the important indicators for judging the degree of fatigue. In this study, the data showed that the BLA levels of the LFT, MFT and HFT groups were significantly

lower compared with that in the control group. The results clearly indicated that TBF can inhibit the production of BLA during exhaustive exercise, and this possibly delays the onset of fatigue.

Serum urea nitrogen (SUN) is the metabolism product of proteins and amino acids (Sun et al., 2014). Urea is formed in the liver as the end product of protein-metabolism and is carried by the blood to the kidneys for excretion. Protein and amino acids have a stronger catabolic metabolism when the body cannot obtain enough energy from sugar and fat catabolic metabolism. There is an inverse correlation between the blood urea nitrogen level in vivo and the exercise tolerance (Wu et al., 2014). Therefore, SUN is another important indicator for evaluating exercise tolerance and fatigue status. In this study, the data showed that the SUN levels of the MFT and HFT groups were significantly lower compared with that in the control group. Reduced SUN levels by MFT and HFT groups reflected reduced protein metabolism, which was indicative of enhanced endurance and ameliorated fatigue (Zhang et al., 2010).

The normal function of creatine kinase (CK) in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. Phosphocreatine is burned as a quick source of energy by cells (Nwose, 2013). However, the normal function of CK is not as relevant, in this case, due to what happens to CK when muscle is damaged (Kim et al., 2012). During the process of muscle degeneration, the muscle cells lyse and their contents find their way into the bloodstream. Because most of the CK in the body normally exists in the muscle, an increase in CK in the blood indicates that muscle damage has occurred or is occurring (Anand et al., 2012). In this study, the data showed that the SCK levels of the HFT groups were significantly lower compared with that in the control group. The results clearly indicated that TBF could ameliorate muscle damage by exhaustion exercise, and the ameliorating effect might contribute to improving exercise endurance capacity (Wang et al., 2006).

Glycogen is an important source of energy during exercise. Liver glycogen depletion might be an important factor in the development of fatigue because as liver glycogen is depleted during exercise, there is an inability to maintain blood glucose level, and the ensuing hypoglycemia could result in impaired nervous function (Shang et al., 2010). In this study, the data showed that the liver glycogen contents of the LFT, MFT and HFT groups were significantly higher compared with that in the control group. Based on these results, it could be concluded that anti-fatigue activity of TBF might be related to the improvement in the metabolic control of exercise and the activation of energy metabolism.

Different hypotheses for physical fatigue were proposed in light of its complexity and involvement of multiple factors (Horng et al., 2014). Over the past two decades, radical theory has been attracting more interest. Radical theory suggests that physical fatigue can develop due to an imbalance between reactive free radicals production and the anti-oxidative defense system of the living body. The accumulation of reactive free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs (Lee et al., 2011). The anti-oxidative defense system of the living body consists of antioxidant enzymes and antioxidant nutrients, which plays an important role in the body's protection against oxidative stress. Principal antioxidant enzymes include SOD, GPx and CAT, and improvement in the activities of these antioxidant enzymes can help to fight against fatigue (You et al., 2011). In this study, the data showed that the SOD, GPx and CAT activities in liver of the MFT and HFT groups were significantly higher compared with that in the control group. The results clearly indicated that TBF could promote increases in the antioxidant enzyme activities in liver, again supporting that TBF has anti-fatigue activity.

Conclusions

In conclusion, the results from the present study showed that TBF prolonged the exhaustive running times of the mice. It sub-served to remove the accumulated products of metabolism by decreasing the levels of BLA and SUN. It ameliorated the muscle damage by decreasing the SCK levels. It improved the metabolic control of exercise and activated the energy metabolism by increasing the liver glycogen contents, as well as improving endogenous cellular antioxidant enzymes in mice by increasing the SOD, GPx and CAT activities. Collectively, these findings indicate that TBF has anti-fatigue activity. However, further study is needed in order to elucidate the more exact mechanism of the anti-fatigue effects of TBF.

http://dx.doi.org/10.4314/ajtcam.v13i2.7

Acknowledgements

This research was supported by the Specialized Research Fund of the Liaoning Normal University (No. 201304891).

References

1. Anand, T., Phani Kumar, G., Pandareesh, M.D., Swamy, M.S. and Khanum, F. (2012). Bawa AS. Effect of bacoside extract from *Bacopa monniera* on physical fatigue induced by forced swimming. Phytotherapy Research, 26(4): 587-593.
2. Azenabor, A.A. and Hoffman-Goetz, L. (1999). Intrathymic and intrasplenic oxidative stress mediates thymocyte and splenocyte damage in acutely exercised mice. Journal of Applied Physiology, **86**: 1823-1827.
3. Ding, J.F., Li, Y.Y., Xu, J.J., Su, X.R. and Gao, X. (2011). Yue FP. Study on effect of jellyfish collagen hydrolysate on anti-fatigue and anti-oxidation. Food Hydrocolloids, **25**(5): 1350-1353.
4. Fabjan, N., Rode, J., Kosir, I.J., Wang, Z. and Zhang, Z.I. (2003). Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. Journal of Agricultural and Food Chemistry, **51**(22): 6452-6455.
5. Gong, F.Y., Li, F.8*/L., Zhang, W.M., Li, J. and Zhang, Z. (2012). Effects of crude flavonoids from tatary buckwheat on alloxan induced oxidative stress in mice. Bangladesh Journal of Pharmacology, **7**: 124-130.
6. Horng, C.T., Huang, J.K., Wang, H.Y., Huang, C.C. and Chen, F.A. (2014). A Antioxidant and antifatigue activities of *Polygonatum Alte-lobatum Hayata* rhizomes in rats. Nutrients, **6**(11): 5327-5337.
7. Jia, Z.S., Tang, M.C. and Wu, J.M. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, **464**: 555-559
8. Kim, N.H., Moon, P.D., Pak, S.C., Kim, H.M. and Jeong, H.J. (2012). Anti-fatigue effect of *Zizania caudiflora* (Turczaninow) Nakai. American Journal of Chinese Medicine, **40**(1): 111-120.
9. Lee, S., You, Y., Yoon, H.G., Kim, K., Park, J., Kim, S., Ho, J.N., Lee, J., Shim, S. and Jun, W. (2011). Fatigue-alleviating effect on mice of an ethanolic extract from *Rubus coreanus*. Bioscience, Biotechnology, and Biochemistry, **75**(2): 349-351.
10. Li, C. and Zhang, L. (2013). *In vivo* anti-fatigue activity of total flavonoids from sweet potato [*Ipomoea batatas* (L.) Lam.] leaf in mice. Indian Journal of Biochemistry and Biophysics, **50**(4): 326-329.
11. Lin, Y., Liu, H.L., Fang, J., Yu, C.H., Xiong, Y.K. and Yuan, K. (2014). Anti-fatigue and vasoprotective effects of quercetin-3-O-gentiobiose on oxidative stress and vascular endothelial dysfunction induced by endurance swimming in rat. Food and Chemical Toxicology, **68**: 290-296.
12. Nwose, C. (2013). Effect of ethanolic leaf extract of *kalanchoe pinnata* on serum creatine kinase in albino rats. Journal of Pharmacognosy and Phytochemistry, **1**(5): 8-11.
13. Qi, B., Liu, L., Zhang, H., Zhou, G.X., Wang, S., Duan, X.Z., Bai, X.Y., Wang, S.M. and Zhao, D.Q. (2014). Anti-fatigue effects of proteins isolated from *Panax Quinquefolium*. Journal of Ethnopharmacology, **153**(2): 430-434.
14. Shang, H.P., Cao, S.H., Wang, J.H., Zheng, H. and Putheti, R. (2010). Glabridin from Chinese herb licorice inhibits fatigue in mice. African Journal of Traditional, Complementary, and Alternative, **7**(1): 17-23.
15. Sun, S., Niu, H., Yang, T., Lin, Q., Luo, F. and. Ma, M. (2014). Antioxidant and anti-fatigue activities of egg white peptides prepared by pepsin digestion. Journal of the Science of Food and Agriculture, **94**(15): 3195-3200.
16. Takeda, K., Machida, M., Kohara, A. and Omi, N. (2011). Takemasa T. Effects of citrulline supplementation on fatigue and exercise performance in mice. Journal of Nutritional Science and Vitaminology, **57**(3): 246-250.
17. Tan, S., Zhou, F., Li, N., Dong, Q., Zhang, X., Ye, X., Guo, J., Chen, B. and Yu, Z. (2013). Anti-fatigue effect of ginsenoside Rb_1 on postoperative fatigue syndrome induced by major small intestinal resection in rat. Biological and Pharmaceutical Bulletin, **36**(10): 1634-1639.

18. Tanaka, M., Ishii, A. and Watanabe, Y. (2013). Neural effect of mental fatigue on physical fatigue: A magnetoencephalography study. Brain Research, **13**: 1383-1388.
19. Wang, J.J., Shieh, M.J., Kuo, S.L., Lee, C.L. and Pan, T.M. (2006). Effect of red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise. Applied Microbiology and Biotechnology, **70**: 247-253.
20. Wu, J.Y., Leung, H.P., Wang, W.Q. and Xu, C. (2014). Mycelial fermentation characteristics and anti-fatigue activities of a Chinese caterpillar fungus, *Ophiocordyceps sinensis* strain Cs-HK1 (Ascomycetes). International Journal of Medicinal Mushrooms, **16**(2): 105-114.
21. Yong-xin, X.. and Jian-jun, Z. (2013). Evaluation of anti-fatigue activity of total saponins of *Radix notoginseng*. Indian Journal of Medical Research, **137**(1): 151-155.
22. You, L., Zhao, M., Regenstein, J.M. and Ren, J. (2011). *In vitro* antioxidant activity and *in vivo* anti-fatigue effect of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. Food Chemistry, **124**: 188-194.
23. Zhang, M., Chen, H., Li, J., Pei, Y. and Liang, Y. (2010). Antioxidant properties of tartary buckwheat extracts as affected by different thermal processing methods. LWT- Food Science and Technology, **43**: 181-185
24. Zhang, X.L., Ren, F., Huang, W., Ding, R.T., Zhou, Q.S. and Liu, X,W. (2010). Anti-fatigue activity of extracts of stem bark from *Acanthopanax senticosus*. Molecules, **16**(1): 28-37.